Claims

1. A method of monitoring cell differentiation comprising:
   (a) culturing cells capable of differentiating into at least one particular cell type containing at least one recombinant nucleic acid molecule comprising a reporter gene encoding a product that is secreted upon cell differentiation, or maintaining a non-human animal comprising such cells, under conditions allowing differentiation of the cells; and
   (b) determining the amount or activity of the reporter gene product either within a body fluid of said transgenic non-human animal or the cell culture medium.

2. The method of claim 1, wherein said recombinant nucleic acid molecule comprises at least one cell type-specific regulatory sequence operably linked to said reporter gene.

3. The method of claim 1 or 2, wherein said cells are or are derived from stem cells.

4. The method of claim 3, wherein said stem cells are embryonic stem cells or multipotent adult progenitor cells (MAPCs).

5. The method of any one of claims 1 to 4, wherein said reporter gene product comprises a secretory leader sequence.

6. The method of any one of claims 2 to 5, wherein said regulatory sequence comprises a promoter and/or enhancer element.

7. The method of any one of claims 1 to 6, wherein said cell type is selected from the group consisting of connecting fibroblasts, stromal cells, endothelial cells, glial cells, neural cells, neuronal cells, hematopoietic cells, smooth muscle cells, skeletal muscle cells, epithelial cells, and cardiac cells.

8. The method of claim 6 or 7, wherein said promoter or enhancer is selected from the group consisting of αMHC, MLC2V, VE-cadherin, Tie-2, Flk-1, Flt-1, GFAP, alpha-1-tubulin and collagen 2 promoter or enhancer.

9. The method of any of claims 1 to 8, wherein said reporter gene product is secreted alkaline phosphatase (SEAP) or alpha-amylase.

10. The method of any one of claims 1 to 9, wherein said recombinant nucleic acid molecule further comprises a selectable marker expressed by multi- or pluripotent cells.

11. The method of any one of claims 1 to 10, wherein said cells form cell aggregates or tissue-like aggregates derived from different cell types.

12. The method of any one of claims 1 to 11, wherein said cells form embryoid bodies (EBs).

13. A reporter gene construct for monitoring cell differentiation comprising a recombinant nucleic acid molecule as defined in any one of claims 1 to 12.

14. A cell as defined in any one of claims 1 to 12 or comprising a reporter gene construct of claim 13, wherein said cell is capable of differentiating into at least one particular cell type.

15. A cell aggregate of at least one cell type obtainable by the method of any one of claims 1 to 12.

16. A tissue obtainable by the method of any one of claims 1 to 12 or comprising cells of claim 14 or a cell aggregate of claim 15.

17. An organ comprising a tissue of claim 16, a cell of claim 14 or a cell aggregate of claim 15.

18. An implant or transplant comprising an organ of claim 17, a tissue of claim 16, a cell of claim 14 or a cell aggregrate of claim 15.

19. A non-human animal comprising a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17.

20. A composition of matter comprising a reporter gene of claim 13, a tissue of claim 16, cells of claim 14 or a cell aggregate of claim 15.

21. An array comprising a solid support and attached thereto or suspended thereon cells of claim 14, a cell aggregate of claim 15 or a tissue of claim 16.

22. An apparatus for analyzing the array of claim 21.

23. A method of obtaining and/or profiling a modulator of cell differentiation comprising:

(a) contacting a test sample comprising a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16 or an organ of claim 17 or a non-human animal of claim 19 with a test substance; and

(b) determining the effect of the test substance on the amount of the reporter gene product or activity compared to a control sample or animal.

24. The method of claim 23, wherein said contacting step further includes contacting said test sample or animal with at least one second test substance in the presence of said first test substance.

25. The method of any one of claims 1 to 12 or 23 to 24, wherein a compound known to activate or inhibit the differentiation process is added to the culture medium or animal.

26. The method of any one of claims 23 to 25, wherein the test substance is a therapeutic agent.

27. The method of any one of claims 23 to 26, wherein the test substance is a mixture of therapeutic agents.

28. The method of any one of claims 23 to 27, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

29. The method of claim 28, wherein said collection of test substances has a diversity of about 10^3 to about 10^5.

30. The method of claim 29, wherein the diversity of said collection of test substances is successively reduced.

31. The method of any one of claims 23 to 30, which is performed on an array.

32. The method of any one of claims 1 to 12 or 23 to 31, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

33. The method of any one of claims 1 to 12 or 23 to 32, wherein said one or more cells or tissue are contained in a container.

34. The method of any one of claims 1 to 12 or 23 to 33, comprising taking 3 or more measurements, optionally at different positions within the container.

35. The method of claim 33 or 34, wherein said container is a well in a microtiter plate.

36. The method of claim 35, wherein said microtiter plate is a 24-, 96-, 384- or 1586-well plate.

37. A method of obtaining and manufacturing a drug which promotes or inhibits formation of specific cell types comprising the steps of any one of claims 23 to 36, wherein an enhanced or reduced level or activity of the reporter gene product is indicative for the drug.

38. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of the method of any one of claims 23 to 37, wherein an enhanced level or activity of the reporter gene product is indicative for said agent.

39. A method of determining toxicity, preferably teratogenicity, embryotoxicity, chronic or acute toxicity of a compound comprising the steps of the method of any one of claims 23 to 37, wherein a reduced or enhanced level or activity of said reporter gene product is indicative for the toxicity of the compound.

40. The method of any one of claims 23 to 39, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

41. The method of any one of claims 23 to 40, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

42. A kit useful for conducting a method of any one of claims 1 to 12 or 23 to 41, containing for example a reporter gene construct of claim 13, a cell of claim 14, and standard compounds, like cell culture media, selection agents, detection agents for the reporter molecule and control samples.

43. A method of conducting a drug discovery business comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying a modulator of cell differentiation; and/or

(b) conducting therapeutic profiling of modulators identified in step (a), or further analogs thereof, for efficacy and toxicity in animals of claim 19; and

(c) formulating a pharmaceutical preparation including one or more modulators identified in step (b) as having an acceptable therapeutic profile.

44. A method of conducting a target discovery business comprising:

(a) providing one or more assay systems of any one of claims 1 to 12 or 23 to 41 for identifying modulators of cell differentiation;

(b) (optionally) conducting therapeutic profiling of modulators identified in step (a) for efficacy and toxicity in animals of claim 19; and

(c) licensing, to a third party, the rights for further drug development and/or sales for modulators identified in step (a), or analogs thereof.

45. A modulator of cell differentiation such as growth and tissue formation promoting identified according to the method of any one of claims 23 to 41.

46. A pharmaceutical composition for use in the modulation of cell differentiation comprising a modulator identified according to the method of any one of claims 23 to 41.

47. A method of making a pharmaceutical composition for use in modulating cell differentiation comprising mixing a modulator of cell differentiation identified according to a method of any one of claims 23 to 41 with a suitable diluent or carrier.

48. Use of a reporter gene construct of claim 13, a cell of claim 14, a cell aggregate of claim 15, a tissue of claim 16, an organ of claim 17, the implant or transplant of claim 18, a non-human animal of claim 19, the composition of claim 20, an array of claim 21 or the apparatus of claim 22 in drug discovery or pharmacokinetic or pharmacological profiling.

49. A vector comprising the promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene, and operably linked thereto a heterologous DNA sequence.

50. The vector of claim 49, wherein said promoter comprises the nucleotide sequence of SEQ ID NO: 1 or 2, or a fragment thereof.

51. The vector of claim 49 or 50, wherein said heterologous DNA sequence encodes a reporter or a selectable marker.

52. The vector of any one of claims 49 to 51, wherein said DNA sequence encodes secreted alkaline phosphatase protein (SEAP).

53. The vector of any one of claims 49 to 52 comprising the nucleotide sequence of SEQ ID NO: 3.

54. Use of a promoter region of the mouse alpha myosin heavy chain gene or of the ventricular myosin regulatory light chain gene for the specific expression of heterologous DNA sequences during embryogenesis or cell development.